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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
A secreted carrier protein has been described which is able to bind to FGF-1 and FGF-2 in a non-covalent, reversible manner. FGF-2 bound to this protein was not subject to degradation and retained its mitogenic activity. This **FGF-binding protein (FGF-BP)** has been studied extensively by our laboratory. FGF-BP is highly expressed in squamous cell carcinomas (SCC) and EGF is able to increase the expression of FGF-BP in SCC derived cell lines through PKC, MEK/ERK, and p38 MAPK signaling. We have found FGF-BP mRNA to be expressed in two breast cancer cell lines (MDA-MB-468, MCF-7/ADR), by Northern Analysis/Ribonuclease Protection. EGF treatment of MDA-MB-468 cells resulted in an increase in FGF-BP mRNA expression in a time-dependent manner. EGF signaling occurs primarily through the PKC, and p38 MAPK pathways, while EGF induction of the FGF-BP promoter is mediated through CCAAT/enhancer binding protein (C/EBP) and AP-1 transcription factor binding sites on the promoter. Finally, overexpression of C/EBPbeta-LAP upregulates FGF-BP promoter activity 80-fold and is reversed with coexpression of C/EBPbeta-LIP, which is mediated primarily through the FGF-BP promoter C/EBP site.

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Annual Summary Report for Grant Number DAMD17-00-1-0265
August 1, 2001 to July 31, 2002

P.I.: Benjamin L. Kagan

Title: Growth Factor Regulation of an Angiogenic Factor, the Fibroblast Growth Factor-Binding Protein (FGF-BP), in Breast Cancer

I. Introduction

Paracrine and autocrine growth factors have many functions, including a crucial role in inducing the formation of new blood vessels in a healing wound, as well as in a growing tumor. Many studies have demonstrated that a solid tumor mass cannot grow beyond a few millimeters in size without a sufficient supply of blood to the tumor. Tumor blood vessels provide a pathway for tumor cells to metastasize to distal sites, as well as a source of nourishment (1-4). The most important and best-studied angiogenesis factors belong to the family of fibroblast growth factors (FGFs) (5, 6). FGF-1 and FGF-2 (aFGF and bFGF, respectively) are unique in that their biological activities can be quenched by binding tightly to heparansulfate proteoglycan molecules in the extracellular matrix (7-10). Two alternate mechanisms of FGF-1 and FGF-2 activation have been theorized as a result of a multitude of studies over the last decade. One mechanism involves the solubilization of FGF-2 from its storage site by heparanase digestion of the glycosaminoglycan portion of the cell attachment (11-14). The second mechanism involves the binding of FGF to a secreted carrier protein delivering the activated FGF to its target receptor. A secreted carrier protein has been described which is able to bind to FGF-1 and FGF-2 in a non-covalent, reversible manner (15). FGF-2 bound to this protein was not subject to degradation and retained its mitogenic activity (15). This **FGF-binding protein (FGF-BP)** has been studied extensively by our laboratory.

Expression of FGF-BP in cell lines that express FGF-2 results in these cells having a tumorigenic and angiogenic phenotype (16). FGF-BP transfected cells have been shown to release the protein into their media along with FGF-2 in a non-covalently bound form; the released FGF-2 is now biologically active (17). FGF-BP mRNA is expressed in SCC, colon, and breast tumor cell lines and primary tumor tissue (16). The role of FGF-BP during tumor progression has been studied by our laboratory using skin carcinogenesis as a model for epithelial cancers. We have shown that FGF-BP mRNA is upregulated in the skin during mouse development, but drops to low levels in adult mouse skin. In both mouse and human skin, FGF-BP mRNA and protein levels increase at least 3-fold upon treatment with PKC-activating TPA (12-*O*-tetradecanoylphorbol-13-acetate), and increase further in DMBA/TPA induced papillomas and carcinomas (18).

II. Body

The human FGF-BP promoter was recently isolated and cloned revealing positive and negative regulatory elements within a 118 base pair region just upstream of the FGF-BP transcription start site (**Figure 1**). The phorbol ester TPA was then shown to upregulate FGF-BP transcription in ME180 squamous cell carcinoma cells. This transcription was mediated through the activation of protein kinase C, and the Sp1, AP-1, and C/EBP positive regulatory elements in the FGF-BP promoter (19). Treatment of ME180 SCC cells resulted in the upregulation of FGF-BP mRNA. Signal transduction was mediated through the EGFR, PKC, MEK/ERK, and p38 pathways, while transcription was mediated through the AP-1 and C/EBP regulatory elements in the promoter (20). Finally, we have also shown that serum upregulates FGF-BP expression in ME180 cells, predominantly through PKC and p38 signaling, while only through the C/EBP site on the FGF-BP promoter (21). **In my accepted proposal, I hypothesized that an angiogenic "funneling" effect exists in which**

intracellular signals intiated by EGF and related ligands result in the activation of FGF through the modulation of the FGF-BP gene. I planned to consider the relevance of this "funneling" effect with respect to the breast cancer system.

We have found FGF-BP mRNA to be expressed in two breast cancer cell lines, 21 of 29 invasive breast cancer samples (**Table 1**), by Northern Analysis/Ribonuclease Protection, and *in situ* hybridization, respectively. We have also detected FGF-BP mRNA in the human and mouse mammary gland. **This report summarizes the findings by Benjamin Kagan as PI of the funded research, testing the role of FGF-BP in human breast cancer cell progression and its regulation by the epidermal growth factor.**

Aim 1: To study the regulation of the FGF-BP mRNA, by growth factors, in breast cancer.

Detection of endogenous FGF-BP mRNA in MCF-7/ADR and MDA-MB-468 human breast cancer cell lines. Previously, we were able to show that FGF-BP mRNA was expressed in 9 out of 15 breast cancer cell lines, by RT-PCR. To study the regulation of FGF-BP expression in breast cancer cell lines, we wanted to use a quantitative method for detection of FGF-BP mRNA. A ribonuclease protection assay specific for human FGF-BP was developed using a riboprobe derived from a pRC/CMV vector plasmid containing the FGF-BP open reading frame (16). We were able to detect FGF-BP mRNA only in the MCF-7/ADR cell line, an adriamycin resistant clone of the MCF-7 cell line, as well as the ME180 SCC cell line, which was used as a positive control. Northern analysis was also used, screening a wider array of breast cancer cell lines (**Figure 2**). **We were able to detect expression of FGF-BP mRNA in both MCF-7/ADR and the MDA-MB-468 cell lines. Expression of FGF-BP mRNA, as determined by RNase protection and Northern analysis, is summarized in Table 1.**

EGF regulation of endogenous FGF-BP in MDA-MB-468 cells. Studies have shown that the MDA-MB-468 cell line overexpresses the EGFR as compared to MCF-7 breast cancer cells (22-24). Biscardi et al. (24) measure levels of EGFR to be 35 fold that of MCF-7 cells. Because the MDA-MB-468 cell line, like the ME180 cell line, express high levels of the EGFR (22), we decided to test whether FGF-BP mRNA expressed in these cells can be regulated by EGF and/or TPA. MDA-MB-468 cells were grown to 80% confluency, serum starved for 24 hours, and treated with EGF for 1, 3, 6, or 24 hours. FGF-BP mRNA levels were analyzed by Northern analysis, and we were able to observe that EGF induced FGF-BP upregulation at about 3-fold above control, peaking at 6 hours of EGF treatment (**Figure 3**). The time-course of EGF induction of FGF-BP mRNA in MDA-MB-468 cells was similar to that observed in the ME180 SCC cell line, suggesting similar mechanisms of regulation (20). **These data demonstrate that EGF can regulate FGF-BP in MDA-MB-468 cells, in a similar manner to ME180 SCC cells**

EGF induction of FGF-BP in MDA-MB-468 cells is mediated through PKC and p38 MAPK signaling. EGF regulation of FGF-BP mRNA in ME-180 cells occurs through PKC, and the MEK/ERK and p38 MAPK signaling pathways (20). Serum, in contrast, mediates FGF-BP transcription through PKC and p38 MAPK signaling, but not MEK/ERK (21). To discern between the possible signaling pathways involved in EGF induction of FGF-BP in MDA-MB-468 cells, we tested pharmacological inhibitors of signal transduction at various concentrations for their affect on FGF-BP regulation. We found that treatment with the EGFR tyrosine kinase inhibitor PD153035 resulted in a significant concentration dependent inhibition of EGF induction of FGF-BP mRNA (**Figure 4**). Therefore, as expected, EGFR tyrosine kinase activity is essential for the EGF effect. To establish whether PKC activation was also required for the EGF effect on FGF-BP, we treated MDA-MB-468 cells with the bisindoylmaleimide PKC inhibitor Ro 31-8220 (25). At concentrations of 1 μM and 10 μM, Ro 31-8220 was able to signficantly inhibit the EGF induction of FGF-BP (**Figure 4**). At these concentrations Ro 31-8220 is also able to inhibit other kinases including the mitogen- and stress-activated protein kinase-1 (MSK1) (26), therefore we tested whether the PKC-specific inhibitor calphostin C (27) could

also inhibit the EGF effect. Treatment with 100 nM calphostin C significantly reduced EGF-induced FGF-BP mRNA expression by 50% (**Figure 4**). **Taken together, these data suggest a role for PKC in the EGF induction of FGF-BP in MDA-MB-468 cells.**

To determine whether different MAP kinase pathways were also involved in the EGF effect on FGF-BP, we used the MEK1/2 specific inhibitor U0126 and the p38 MAPK specific inhibitor SB202190 (28, 29). Treatment with 1 μM and 10 μM U0126 did not significantly inhibit EGF induction (**Figure 4**). Although 20 μM U0126 significantly inhibited EGF induction of FGF-BP, the overall inhibition was only around 30% as compared to the ability of U0126 to inhibit the EGF induction of FGF-BP in ME-180 cells by 70% (20). This suggests a lesser role for the MEK/ERK pathway in the EGF effect in MDA-MB-468 cells. In contrast, as seen in the ME-180 cells, treatment with increasing concentrations of the p38 MAPK inhibitor SB202190, resulted in a concentration-dependent inhibition of EGF-induced FGF-BP mRNA expression ranging from 55% inhibition at 5 μM to 80% inhibition at 20 μM. Furthermore, as described above, the bisindoylmaleimide Ro 31-8220 was able to significantly inhibit EGF-induced FGF-BP mRNA expression at concentrations specific for PKC and other kinases such as MSK1. MSK1 has been shown to be activated by p38 MAPK phosporylation (26, 30). **Taken together, these data suggest that p38 MAPK plays a dominant role in the induction of FGF-BP by EGF in MDA-MB-468 cells.**

Other intracellular targets for EGF receptor-induced intracellular signaling include members of the c-Src protein tyrosine kinase family. c-Src family members interact with the EGFR at tyrosine residues via SH2 domains (31). MDA-MB-468 cells have been shown to express moderate levels of c-Src protein as compared normal breast epithelium (24). Therefore, we used the c-Src family specific inhibitor PP1 (32). Treatment with PP1 resulted in a maximal inhibition of EGF induction of FGF-BP of 20% only at the highest concentration, 10 μM (**Figure 4**). Concentrations of 1 μM and 0.1 μM, also shown to inhibit s-Src family members (32), had no effect. This suggests that c-Src family members do not play a role in the EGF effect. **These results are included in a manscript submitted for publication with Benjamin Kagan as first author (see Reportable Outcomes).**

Aim 2: To study the regulation of the human FGF-BP promoter in breast cancer cells.

EGF regulation of the FGF-BP promoter in MDA-MB-468 cells. As described above, EGF induces the upregulation of FGF-BP in MDA-MB-468 breast cancer cells. To determine if this regulation occurred at the transcriptional level, we tested whether EGF regulated the activity of FGF-BP promoter in MDA-MB-468 cells. As described above, various portions of the human FGF-BP promoter, full-length, mutated, or deleted, have been cloned upstream of a luciferase reporter gene. These constructs have been used successfully to assess the activity of the FGF-BP promoter in ME180 cells (19, 20, 33). We were able to show that in MDA-MB-468 cells, treatment with EGF was able to induce the activity of the –1060/+62 and –118/+62 promoter constructs 4- to 5-fold above basal (**Figure 5**). Deletion of either the AP-1 or the C/EBP, and not the Sp1(b) site, reduced the induction by EGF of the promoter constructs, suggesting the AP-1 and the C/EBP sites were necessary for EGF induced FGF-BP transcription in this cell line. This observation is similar to what was observed in the ME180 cells (20). Upon further investigation, cell-type specific differences were observed. Deletion of the AP-1 site resulted in a statistically significant decrease in promoter basal activity, suggesting the AP-1 site is necessary for basal activity. Deletion of the C/EBP site revealed a statistically significant increase in promoter basal activity, suggesting differences in C/EBP binding to the site affecting both basal and EGF induced activity of the FGF-BP promoter. **These data show that EGF is able to induce the activity of the FGF-BP promoter in MDA-MB-468 cells, through the AP-1 and C/EBP sites, as seen in ME180 cells. In addition, C/EBP binding to the FGF-BP promoter may repress basal activity while enhancing promoter activity after EGF treatment.**

C/EBPβ isoform regulation of FGF-BP promoter activity in MDA-MB-468 cells. As shown in Figure 5, deletion of the C/EBP site on the FGF-BP promoter results in a significant increase in basal promoter

activity, in MDA-MB-468 cells. This suggests that a transcription factor complex that binds to this site might act as a repressor, reducing the basal activity of the FGF-BP promoter, which is a unique aspect of regulation of FGF-BP promoter activity not previously observed. Recently, a variant of C/EBPβ, the C/EBPβ-liver enriched inhibitory protein (LIP), translated from the same mRNA as the full length protein but at a downstream start codon (also called C/EBPβ-liver enriched activating protein or LAP), has been described (34, 35). The LIP variant is similar to LAP, except that it does not contain a transactivating domain. The C/EBPβ-LIP –LAP dimer is able to bind to its normal consensus site on a promoter, with greater affinity than LAP-LAP dimers, but is not able to promote transcription, therefore acting as a dominant negative (34). To determine whether differences in C/EBPβ isoform expression could account for the tissue-specific variations observed, we compared the levels of C/EBPβ isoform expression in MDA-MB-468 (where the C/EBP site is repressive) and ME-180 cells (where the C/EBP site has no impact on basal activity). Immunoblot analysis using an antibody specific for the carboxyl-terminus of C/EBPβ revealed that MDA-MB-468 cells express higher levels of LIP relative to LAP **(Figure 6A, 20 kd band)** as compared to ME-180 cells. In fact, as shown in **Figure 6B**, ME-180 cells have a 3-4 fold higher LAP/LIP ratio than MDA-MB-468 cells and **we conjectured that these differences in LAP/LIP ratios might explain the differences in basal activity seen with the ΔC/EBP promoter construct ((20) and Figure 5).**

Effects of C/EBPβ isoforms on the activity of the FGF-BP promoter. C/EBPβ-LIP has been shown to have a higher DNA-binding affinity than LAP, and that small increases in the levels of LIP present, relative to levels of LAP, confer a significant increase in the ability of transcriptional repression to occur (34). These results are consistent with the notion that the ratio of LAP to LIP present in a cell is an important factor that determines the transactivating ability of a C/EBPβ complex. To determine whether changes in C/EBPβ isoforms levels impacted on the activity of the FGF-BP promoter, we transiently co-transfected expression vectors for either LAP alone or LAP and LIP together (CMV-LAP, CMV-LIP, respectively) along with FGF-BP promoter constructs in MDA-MB-468 cells. Overexpression of LAP alone resulted in a significant increase of 80-fold in the basal activity of the -118/+62 FGF-BP promoter construct (**Figure 7A**). In contrast, overexpression of LAP in ME-180 cells resulted in a much lesser 8-fold increase in basal activity of the -118/+62 construct.

The expression vector CMV-LIP was then introduced into MDA-MB-468 cells in increasing concentrations (2 μg and 4 μg), along with constant amounts of CMV-LAP (5 μg) and FGF-BP promoter constructs, to ascertain the effects of decreasing the ratio of LAP to LIP on the FGF-BP promoter. Co-transfection of 2 μg of LIP along with LAP, conferring a LAP/LIP ratio of approximately 2 (**Figure 7B**) and this change in LIP/LAP ratio reduced the superinduction of the -118/+62 construct by 50% (**Figure 7C**). Increasing LIP to 4 μg, conferring a LAP/LIP ratio of approximately 1 (Figure 7B), and this significantly decreased the superinductive effect of LAP to a level 70% below the level of induction seen with LAP alone (Figure 7C). The effects observed due to the changes in LAP to LIP ratio were primarily limited to the C/EBP site, because transfection with the ΔC/EBP construct in a constant activity level 80% below the level seen with the -118/+62 construct (Figure 7C, inset).

Binding of C/EBPβ-LAP and -LIP to the C/EBP site on the FGF-BP promoter. To further investigate the interactions of C/EBPβ-LIP and -LAP with the C/EBP site we performed gel mobility shift analysis. As shown in **Figure 8** (lanes 1 and 9), incubation of nuclear extracts from untreated and EGF-treated MDA-MB-468 with the radiolabeled -55/-30 oligonucleotide probe resulted in the formation of four complexes a- d. Although, it should be noted that frequently in different preparations of nuclear extracts, one or more of the complexes was indistinguishable and EGF treatment of cells resulted in no consistent differences in the mobility or the amount of any of the complexes (Figure 8 lanes 1,2 vs 9,10). To improve the resolution and to help identify components of these complexes, we examined binding to the FGF-BP promoter C/EBP element in extracts from cells in which levels of LIP or LAP had been increased by transient overexpression. Increasing levels of LIP, which functionally results in FGF-BP promoter repression (Figure 7C), enhanced both the c and d complexes in the untreated and EGF-treated extracts (Figure 8 lanes 3 and 11). In contrast, the overexpression

of LAP significantly increased the b complex and also slightly increased the c complex (Figure 8 lanes 6 and 14). We also determined that an antibody to C/EBP supershifted the b, c and d complexes produced after overexpression of LIP or LAP (Figure 8 lanes 5, 8, 13 and 16).

The next question was then to determine how EGF and p38 MAPK signaling affected the binding of these complexes. Under conditions of basal levels of LIP and LAP, inhibition of p38 MAPK produced no consistent change in complex binding either in the presence or absence of EGF, although again in many of the extracts the levels of binding to the complexes were low and somewhat variable. Furthermore, under conditions of LIP overexpression, inhibition of p38 MAPK had little effect on the d complex (Figure 8 lanes 4 and 12). In contrast, inhibition of p38 MAPK with SB202190 decreased the LAP- induced binding of the c complex in both basal and EGF-treated conditions (Figure 8 lanes 7 versus 15). These data suggest that increased binding of the c complex can be induced by activation of the p38 MAPK signaling cascade. Interestingly, the binding of the b complex is differentially regulated under basal versus EGF-induced conditions. If EGF is present then inhibition of the p38 MAPK increases binding of complex b (Figure 8 lanes 14 versus 15) whereas in the absence of EGF, inhibition of p38 MAPK decreases binding of b (Figure 8 lane 6 versus 7). **This suggests that other EGF-induced signaling pathways can alter the susceptibility of the b complex to regulation by p38 MAPK. In summary it seems that basal repression of the promoter by LIP is well correlated with the formation of the d complex. The effect of p38 MAPK is mainly on the b and c complexes, the DNA binding of which can be induced by p38 MAPK signaling especially by ongoing EGF stimulation. These results are also included in a submitted manscript with Benjamin Kagan as first author.**

Aim 3: To study the role of HER2 in the regulation of the human FGF-BP gene in breast cancer.

The basis of Aim 3 depended on the expression of FGF-BP in MCF-7 breast cancer cells. As stated above, FGF-BP was only detected in two human breast cancer cell lines. The MCF-7/Adr cell line is a clonal variant of the parental MCF-7 cell line but does not exhibit characteristics of its parental lineage. Because FGF-BP mRNA was not detected in MCF-7 cells, the role of HER2 in the regulation of FGF-BP transcription could not be pursued. Therefore the majority of this study focused on the effects of growth factors, specifically EGF, on the regulation of FGF-BP mRNA transcription in the MDA-MB-468 cell line, of which the results are described in Aims 1 and 2.

III. Key Research Accomplishments

- Expression of FGF-BP mRNA was detected in both MCF-7/ADR and the MDA-MB-468 cell lines by Northern analysis and RNase protection.
- EGF upregulates FGF-BP expression in MDA-MB-468 cells, in a similar manner to ME180 SCC cells. This occurs predominantly through the PKC and p38 MAPK signaling pathways.
- EGF is able to induce the activity of the FGF-BP promoter in MDA-MB-468 cells, through the AP-1 and C/EBP sites, as seen in ME180 cells. Deletion of the C/EBP site on the FGF-BP promoter results in a significant increase in basal promoter activity.
- C/EBPβ-LAP and –LIP are expressed at equal levels in MDA-MB-468 cells.
- Overexpression of LAP increases FGF-BP promoter basal activity by 80-fold which is reversed as a result of co-expression with LIP.
- EGF affects the binding of LAP and LIP-containing transcriptional complexes to the C/EBP site on the FGF-BP promoter in manner dependent on p38 MAPK.

IV. Reportable Outcomes

Manuscripts, abstracts, and publications produced as a result of this funded research:

Kagan BL, Harris VK, Coticchia CM, Ray R, Wellstein A and Riegel AT, Transcriptional regulation of a binding protein for FGF (FGF-BP) through p38/SAPK2 signaling. In: *Proceedings of the American Association for Cancer Research, New Orleans, LA,* March 24-March 28, *2001.*

Kagan BL, WellsteinA, and Riegel AT, The roles of p38 MAPK and CCAAT/enhancer-binding protein β in the transcriptional regulation of the fibroblast growth factor-binding protein in breas cancer. In: *Proceedings of the American Association for Cancer Research, San Francisco, CA*, April 6-10, *2002*

Kagan BL, Cabal-Manzano R, Stoica GE, Nguyen Q, Wellstein A, and Riegel AT, Complex regulation of the fibroblast growth factor-binding protein (FGF-BP) in MDA-MB-468 breast cancer cells by CCAAT/enhancer-binding protein β. *2002* (manuscript submitted)

Degrees obtained supported by this award:

Benjamin L. Kagan, Ph.D., Pharmacology, Georgetown University, conferred July 31, 2002

Employment applied for based on work supported by this award:

Benjamin L. Kagan, NIGMS/NIH Pharmacology Research Assistant Training (PRAT) Post-doctoral fellowship in the laboratory of S. Stoney Simmons, Jr., Ph.D., NIDDK/NIH starting 9/30/2002

V. Conclusions

In conclusion, this study characterizes the signal transduction and transcriptional mechanisms that are important in the basal control and EGF induction of FGF-BP expression in breast cancer cells. Understanding the regulatory pathways involved in the expression of FGF-BP is important, especially because deregulated FGF-BP expression is rate-limiting in tumor formation and tumor angiogenesis (16, 17). We have now observed a link between EGF signaling and FGF-BP gene induction in both SCC (20) and breast cancer cells involving p38 MAPK signaling via AP-1 and C/EBP induced transcription. Although the phosphorylation site of C/EBPβ in response to p38 MAPK has not been defined *in vivo*, it is tempting to speculate that p38 MAPK can directly phosphorylate C/EBPβ and thus activate this molecule. We have also demonstrated the ability for the differential binding of C/EBP factors, specificly C/EBPβ-LAP and LIP, to regulate both unstimulated and EGF-stimulated promoter activity of the human FGF-BP gene in human breast cancer cells. Although many studies demonstrate that p38 MAPK has role in angiogenesis (36) and the regulation of expression of angiogenic factors such as VEGF (37), a role for C/EBP factors in the process of angiogenesis is not well characterized. This study suggests that along with its well-defined role in mammary gland development, C/EBPβ may well play a role in the pathology of breast cancer, particularly through the control of angiogenesis in the invasive phenotype.

Appendix:

Figure 1. Regulatory region of the FGF-BP promoter

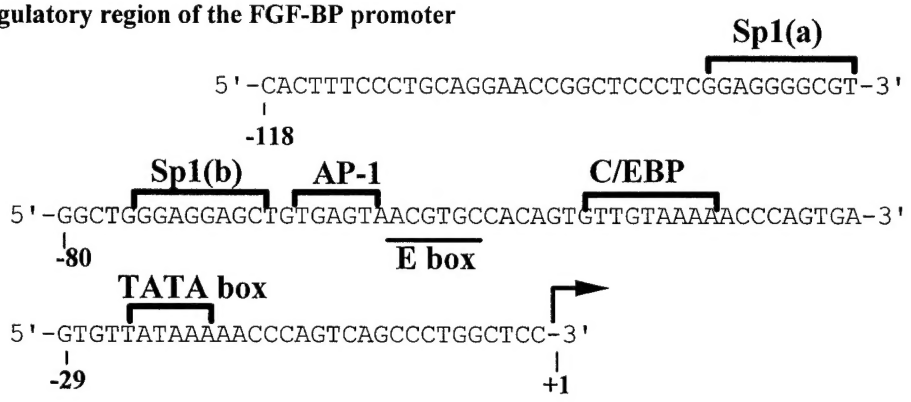


Table 1. Levels of FGF-BP mRNA in human breast cancer samples as detected by *in situ* hybridization.

	FGF-BP expression		
	+	-	
Ductal in situ	1	12	p = 0.0008
Ductal invasive	15	8	
Lobular in situ	2	8	p = 0.0075
Lobular invasive	6	1	
All in situ	3	20	p < 0.0001
All invasive	21	9	

Figure 2. Levels of FGF-BP as detected by Northern analysis in human breast cancer cell lines.

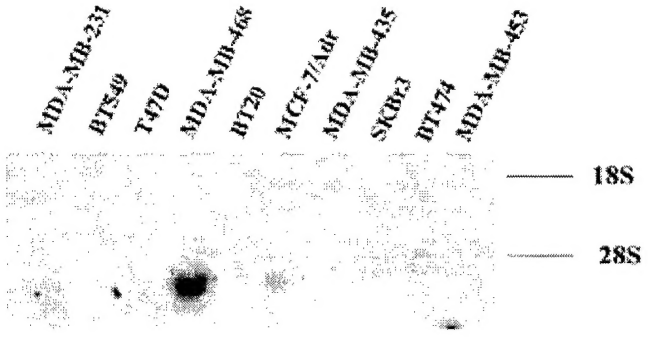


Figure 3. EGF induction of FGF-BP mRNA in MDA-MB-468 cells.

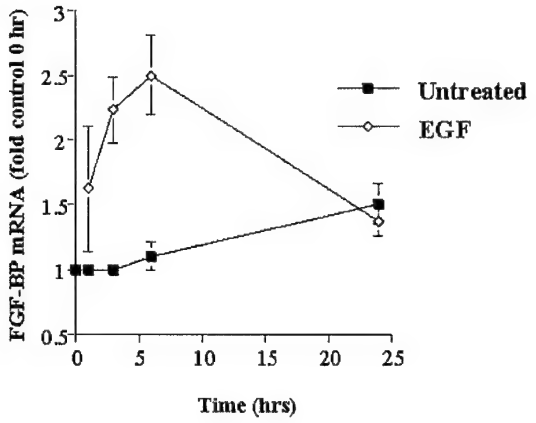


Figure 4. Effect of signal transduction inhibitors on the EGF induction of FGF-BP in MDA-MB-468 cells. The following inhibitors were used: Calphostin C (PKC), PD153035 (EGFR), U0126 (MEK1/2), PP1 (c-Src), Ro 31-8220 (PKC, MSK1), and SB202190 (p38/MAPK).

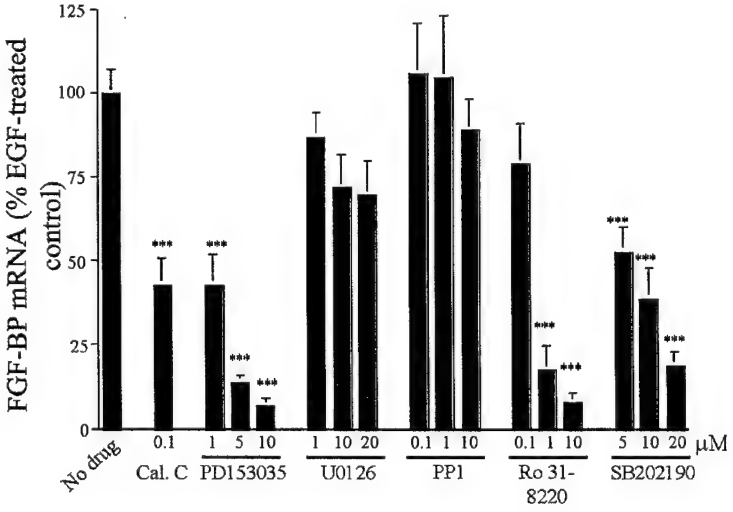


Figure 5. Elements necessary for basal and EGF-inducing activity on the FGF-BP promoter in MDA-MB-468 cells.

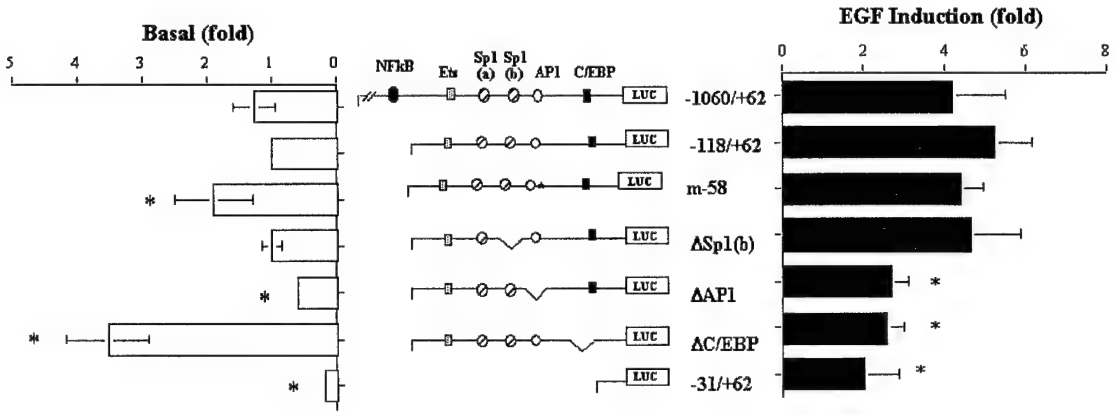


Figure 6. Expression of C/EBPβ in ME-180 and MDA-MB-468 cells. *A,* Western blot analysis of C/EBPβ protein levels in nuclear extracts from untreated or EGF-treated ME-180 and MDA-MB-468 cells. *B,* Ratios of LAP to LIP in MDA-MB-468 and ME-180 cells.

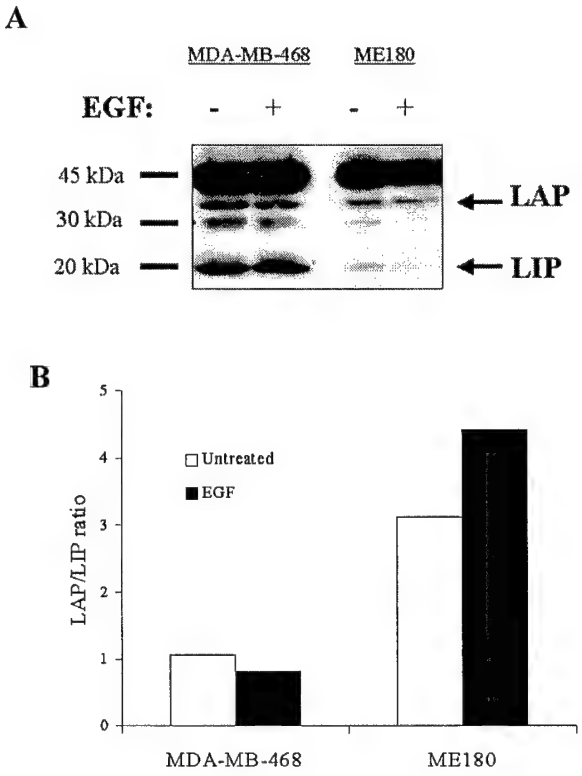


Figure 7. Effects of LAP and LIP overexpression on FGF-BP promoter activity. *A,* MDA-MB-468 cells and ME-180 cells were cotransfected with the indicated FGF-BP promoter constructs and 5 μg CMV-LAP. *B,* Shown is a representative Western blot of C/EBPβ protein levels in MDA-MB-468 cells transiently transfected with CMV-LIP and CMV-LAP. *C,* MDA-MB-468 cells were co-transfected with the -118/+62 or ΔC/EBP (*C, inset*) FGF-BP promoter constructs, 5 μg CMV-LAP and the indicated amounts of CMV-LIP.

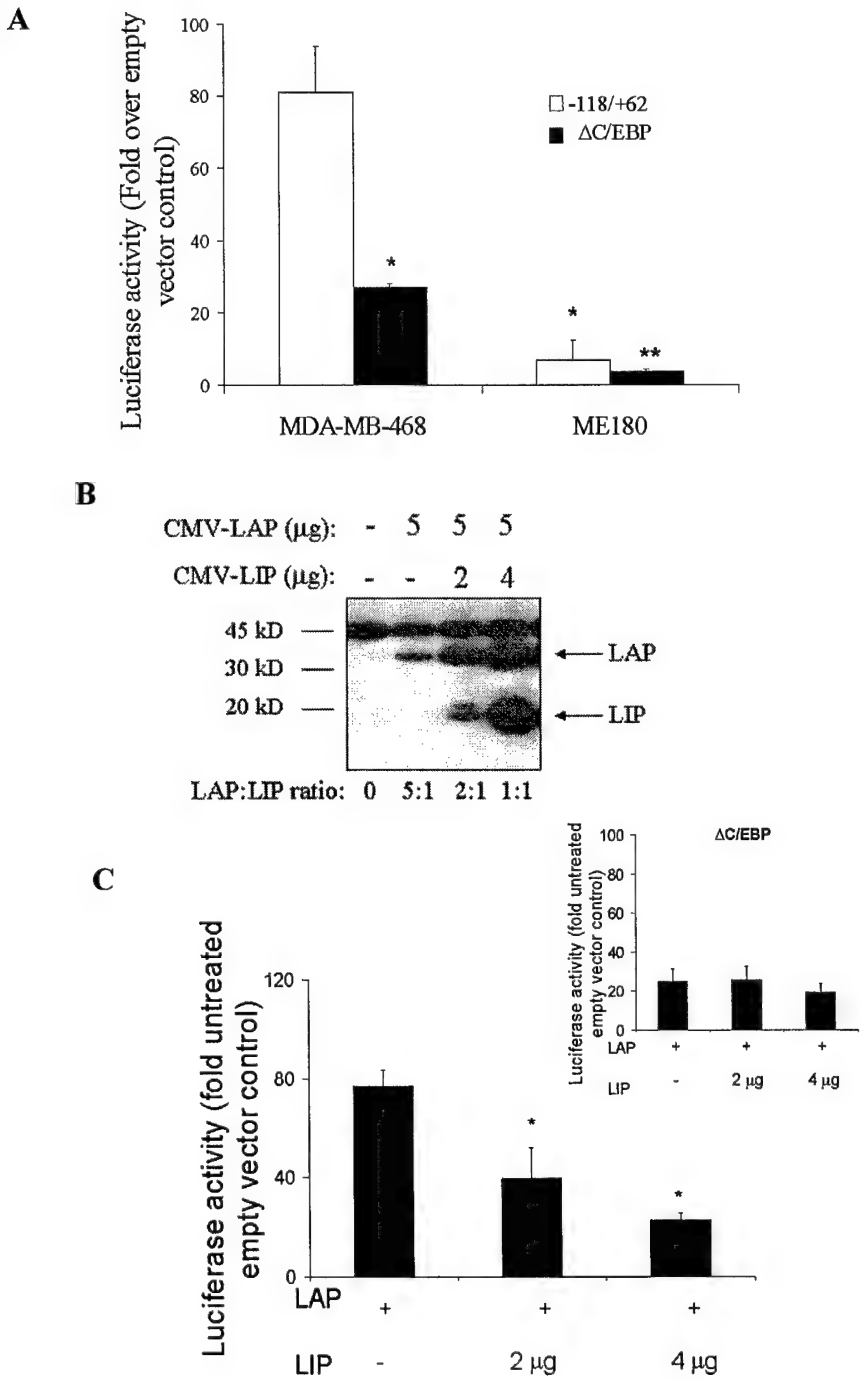
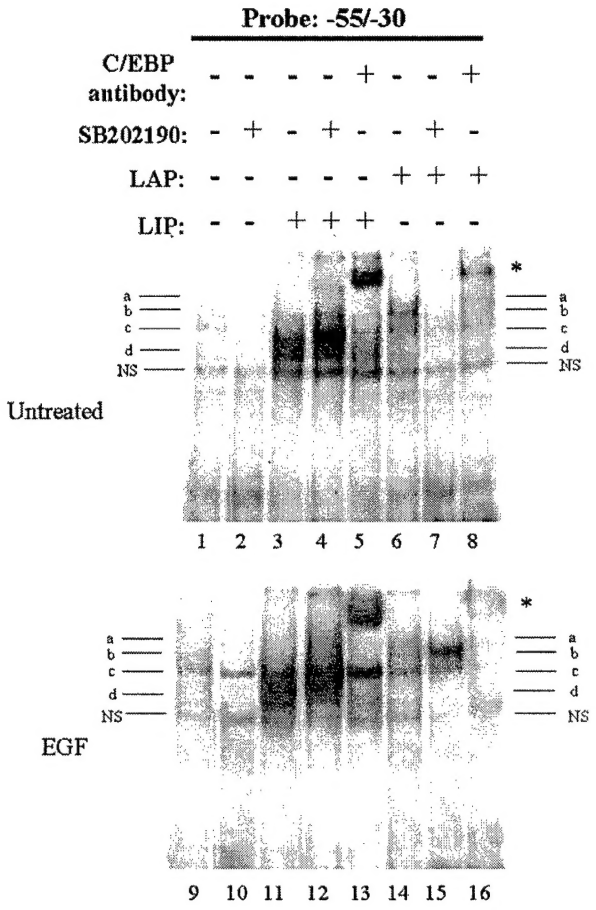


Figure 8. Binding of C/EBPβ-LAP and –LIP to the C/EBP site on the FGF-BP promoter. Radiolabeled -55/-30 FGF-BP promoter sequence was incubated in the presence of nuclear extracts of MDA-MB-468 cells transfected with: empty vector, CMV-LAP, or CMV-LIP constructs. Cells were pretreated with or without p38 MAPK or MEK1/2 inhibitors for 1 h, and were treated with or without EGF for 1 hour.



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